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Betaine Distribution in the Euphorbiaceae

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توزيع البيتايين في العائلة إيوفوربييسـي

تم فحص الأجزاء الهوائية لسبعة عشر نوعا من العائلة اللبـيّنـية (إيوفوربييسـي) موزعة على أحد عشر جنسا، وذلك للكشف عن وجود مركبات البيتايين. وقد تم فصل مركب ترايجونيلين من جميع الأنواع المدروسة، ولكن بمحتوى منخفض يتراوح من ٠,٠٠٤ % (بناء على الوزن الجاف) من ايوفوربيا تيروكالي إلى ٠,١ % من الخروع. وكذلك وجدت مادة جلايسين بيتايين في عديد من نباتات الدراسة بإنتاجية تتراوح من ٠,٠٠٤% من إيوفوربيا تريا نجولارس و كاميسيس هيرتا إلى ١% من جاتروفا مالتيفيدا و جاتروفا بوداجريكا. ومع أنه تم فحص عدد محدود من الأنواع، يمكن الاستنتاج أن العائلة اللبـيّنـية (إيوفوربييسـي) يمكن تصنفيها كفصيلة غير مراكمة للبيتايين.

Aerial parts of 17 species of Euphorbiaceae, distributed in 11 genera, have been examined for the presence of betaines. Trigonelline was isolated from all the tested species, but the yield was low, varying from 0.004 % (based on dry weight) from *Euphorbia tirucalli* to 0.1 % from *Ricinus communis*. Glycinebetaine was also found in many of the plants examined, the yield varying from 0.004 % from *Euphorbia triangularis* and *Chamaesyce hirta* to 0.1 % from *Jatropha multifida* and *J. podagrica* Although only a restricted number of species has been examined, the conclusion can be drawn that Euphorbiaceae can be classified as a non-betaine accumulating family.

Key words: Euphorbiaceae, betaines, glycinebetaine, trigonelline, non-betaine accumulating family.

INTRODUCTION

The most studied role of betaines is that of compatible cytoplasmic solutes aiding adaptation to saline and dry conditions. For this use, however, the concentrations of the compounds must be high, as in the Chenopodiaceae (Adrian-Romero *et al.* 1998) and Amaranthaceae (Blunden *et al.* 1999), but in many betaine-containing plants,

the levels of these compounds are low, as in the Polygonaceae (Adrian-Romero *et al.* 1998) and Bromeliaceae (Adrian-Romero & Blunden 2001). In recent years it has been demonstrated that betaines have a role in aiding plants to resist attack by pathogens. Application of betaines in low amounts to plants has resulted in significant enhancement of the ability of the treated plants to resist attack by fungi (Kraska & Schönbeck 1992; Manninger *et al.* 1992) and root knot nematodes (Jenkins *et al.* 1998).

Systematic studies to determine the distribution of betaines in the plant kingdom have been restricted and directed mainly towards herbaceous species of saline and dry habitats (Rhodes & Hanson 1993). We have been studying both the qualitative and quantitative distribution of these compounds in the plant kingdom and Angiosperm families examined to date include Lamiaceae/ Labiatae (Blunden *et al.* 1996), Capparaceae (McLean *et al.* 1996), Amaranthaceae (Blunden *et al.* 1999), Bromeliaceae (Adrian-Romero *et al.* 2001) and Malvaceae (Blunden *et al.* 2001). In this present study we report on the qualitative and quantitative distribution of these compounds in some species of Euphorbiaceae. Previously trigonelline was reported in *Euphorbia paralias* (Wyn Jones & Storey 1981). In this species, as well as in *E. peplus*, Adrian-Romero *et al.* (1998) reported glycinebetaine, but not trigonelline.

MATERIALS AND METHODS

The species examined and their places of collection are listed in Table 1. Voucher samples of all the species collected in the United Kingdom, Spain, South Africa and Swaziland are maintained in the herbarium of the Hampshire County Council Museums Service, Winchester, Hampshire, U.K. (Index Herbariorum code HCMS; accession number Bi 2000. 16). The voucher specimens of the species collected in Venezuela are lodged in the herbarium of the Faculty of Pharmacy, University of Los Andes, Mérida, Venezuela (Index Herbariorum code MERF).

Only aerial parts of the plants were collected. The plant material was either air dried (samples from Spain, Venezuela, South Africa and Swaziland) or oven dried at 60^{o}C (British samples), powdered and extracted (0.5-4 g) with 80 % methanol. After recovery of the methanol, the aqueous extract was applied to a column of Amberlite IR-120 resin (H^+), which was washed with water prior to elution of the betaines with ammonia solution (Blunden *et al.* 1981). The eluate was evaporated to dryness, the residue dissolved in D_2O and examined by thin layer chromatography (TLC) and ^{1}H NMR spectroscopy (400 MHz). The content of each betaine present in the extracts was estimated using the ^{1}H NMR spectroscopic assay method of Blunden *et al.* (1986).

RESULTS AND DISCUSSION

The Euphorbiaceae was divided into the five subfamilies, Phyllanthoideae, Oldfieldioideae, Acalyphoideae, Crotonoideae and Euphorbioideae, by Webster (1975); these were, in turn, subdivided into 13, 4, 19, 11 and 5 tribes, respectively. Several of these tribes are also split into sub-tribes. For this present study, extracts were prepared of the aerial parts of 17 species, included in 11 genera, which are located in 4 of the 5 subfamilies of Euphorbiaceae. No representative of Oldfieldioideae is included.

Each extract was tested for the presence of betaines, which were not detected by TLC in most of the extracts, even after partial purification. However, when examined by ^{1}H NMR spectroscopy, the presence of trigonelline (N -CH_3 resonance at δ 4.33) was demonstrated in every extract and glycinebetaine {N -$(CH_3)_3$ resonance at δ 3.37} in those of many (Table 1).

The betaine yields were consistently low. The highest yield of trigonelline was 0.1 % from *Ricinus communis*, but for all the other species tested the yield was in the range of 0.004 to 0.08 %. Glycinebetaine contents were also low. The highest yield was from the two *Jatropha* species examined, *J. multifida* and *J. podagrica* (0.1 %) and the lowest from *E. triangularis* and *Chamaesyce hirta* (0.004 %).

Table 1. Betaine distribution in the Euphorbiaceae

Species	Place of collection	Date of collection	Trigonelline yield (% dry weight)	Glycinebetaine yield (% dry weight)
PHYLLANTHOIDEAE *Phyllanthus niruri* L.	Medicinal Plant Garden, Mérida, Venezuela	November 2001	0.03	0.01
ACALYPHOIDEAE *Ricinus communis* L.	La Herradura, Granada, Spain	October 2000	0.1	nd
Mercurialis biennis L.	Near Lambourn, Berkshire, UK	April 2001	0.06	0.03
Acalypha alopecuroidea Jacq.	Medicinal Plant Garden, Mérida, Venezuela	November 2001	0.01	nd
CROTONOIDEAE *Cnidosculus urens* (L.) Arthur	Lagunillas-Chiguará, Mérida State, Venezuela	November 2001	0.01	0.005

Table 1 (*continued*)

Jatropha multifida L.	Near Manzini, Swaziland	February 2002	0.03	0.1
Jatropha podagrica Hook.	Honey Hill, near Manzini, Swaziland	February 2002	0.005	0.1
Manihot esculenta Crantz	Near Manzini, Swaziland	February 2002	0.07	nd
EUPHORBIOIDEAE *Chamaesyce hirta* (L.) Millsp	Medicinal Plant Garden, Mérida, Venezuela	November 2001	0.02	0.004
Euphorbia clavarioides Boiss.	Isandlwana, KwaZulu Natal, South Africa	February 2002	0.01	nd
Euphorbia cotinifolia L.	Medicinal Plant Garden, Mérida, Venezuela	November 2001	0.01	0.03
Euphorbia ingens E. Mey.	Babanango Valley, KwaZulu Natal, South Africa	February 2002	0.02	nd
Euphorbia peplus L.	Havant, Hampshire, UK	June 2001	0.005	0.006
Euphorbia tirucalli L.	Near Manzini, Swaziland	February 2002	0.004	0.02
Euphorbia triangularis Desf.	Babanango Valley, KwaZulu Natal, South Africa	February 2002	0.05	0.004
Pedilanthus tithymaloides (L.) Poit. *P. tithymaloides*	Near Manzini, Swaziland Medicinal Plant Garden, Mérida, Venezuela	February 2002 November 2001	0.02 0.03	0.04 nd
Spirostachys africanus Sond.	Babanango Valley, KwaZulu Natal, South Africa	February 2002	0.08	nd

nd=not detected

Several species of *Euphorbia* were included in the study, some of which yielded both trigonelline and glycinebetaine (*E. peplus*, *E. cotinifolia*, *E. triangularis* and *E. tirucalli*), but for others (*E. clavarioides* and *E. ingens*), only trigonelline was detected. In an earlier report, only glycinebetaine was recorded for *E. peplus* (Adrian-Romero *et al.* 1998), but in this current study, trigonelline was also found. Two samples of *P. tithymaloides* were examined, one collected in Swaziland and

one in Venezuela. Whereas the latter contained both glycinebetaine and trigonelline, the Swazi material yielded only trigonelline.

Rhodes & Hanson (1993) classified the betaine-yielding families and genera into those that accumulate betaines and those that do not. Although only a restricted number of species in the family have been tested, the results obtained show that Euphorbiaceae should be included in the non-betaine accumulating group.

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